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Cloning the *RAD51* homologue of *Schizosaccharomyces pombe*

Daan F.R.Muris, Kees Vreeken, Antony M.Carr[1], Bernard C.Broughton[1], Alan R.Lehmann[1], Paul H.M.Lohman and Albert Pastink*

Medical Genetics Centre South-West Netherlands, Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, The Netherlands and [1]MRC Cell Mutation Unit, Falmer, Brighton, East Sussex BN1 9RR, UK

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ABSTRACT

The *RAD51* gene of *Saccharomyces cerevisiae* encodes a *RecA* like protein, which is involved in the recombinational repair of double strand breaks. We have isolated the *RAD51* homologue, *rhp51*⁺, of the distantly releated yeast strain *Schizosaccharomyces pombe* by heterologous hybridization. DNA sequence analysis of the *rhp51*⁺ gene revealed an open reading frame of 365 amino acids. Comparison of the amino acid sequences of *RAD51* and *rhp51*⁺ showed a high level of conservation: 69% identical amino acids. There are two *MluI* sites in the upstream region which may be associated with cell cycle regulation of the *rhp51*⁺ gene. The *rhp51*⁺ null allele, constructed by disruption of the coding region, is extremely sensitive to X-rays, indicating that the *rhp51*⁺ gene, like *RAD51*, is also involved in the repair of X-ray damage. The structural and functional homology between *rhp51*⁺ and *RAD51* suggests evolutionary conservation of certain steps in the recombinational repair pathway.

INTRODUCTION

The yeast *Saccharomyces cerevisiae* has been used extensively to study DNA-repair mechanisms. Repair deficiency mutations in this organism have been assigned to three epistasis groups, which are thought to represent three different DNA-repair pathways. The *RAD3* group genes are involved in nucleotide excision repair (NER). Mutants in this group are characterized by a high sensitivity to UV-radiation. The *RAD6* group genes are required for resistance to both UV- and ionizing-radiation. Some of the genes belonging to this group are involved in error-prone repair. The *RAD52* group genes are sensitive to ionizing-radiation but not to UV, and they are involved in recombinational repair of double strand breaks (DSBs) (for reviews see 1,2,3).

In case of the *RAD6* gene, which codes for a ubiquitin conjugating enzyme, structural and functional homologues have been isolated from man, *Drosophila melanogaster* and *Schizosaccharomyces pombe* (4,5,6). Several genes involved in the NER mechanism in yeast are also evolutionary conserved. The *RAD3* gene, the *S.pombe rad15* and the human *ERCC2* gene, which is defective in Xeroderma pigmentosum (XP) group D cells are structural homologues (7). In case of the human *ERCC3* gene, which complements XP-B cells, closely related genes have been isolated from *S.cerevisiae* (8), *S.pombe* (9) and *D.melanogaster* (10,11). In addition, the *S.cerevisiae RAD2*, *RAD10* and *RAD14* genes are the homologues of the human *ERCC5*, *ERCC1* and *XPAC* genes, respectively (12,13,14) and the *RAD2* and *RAD10* genes also have homologues in *S.pombe* (15,12) as does the *RAD1* gene (16). The high level of identical amino acids between several proteins involved in NER in both yeasts and man, strongly suggests that this repair pathway is conserved in both species.

Much less is known about the conservation of the *RAD52* group, which constitutes the genes *RAD50* to *RAD57*. The *RAD52* group genes are involved in the recombinational repair of DSBs in DNA, which arise as intermediates during normal cellular processes or are induced by DNA-damaging agents. All mutants of the *RAD52* group have been isolated on the basis of their sensitivity towards ionizing-radiation and methyl methanesulfonate (MMS). Among the *RAD52* group, mutations in *RAD51*, *52* and *54* confer the most extreme sensitivity. These three mutants are defective in induced mitotic recombination and in mating-type switching and *rad51* and *52* mutants are also defective in meiotic recombination, as manifested by the formation of inviable spores. In *rad51*, *52* and *54* mutants the repair of DSBs is almost completely blocked (2).

Recently, the characterization of the *RAD51* gene of *S.cerevisiae* has been reported (17,18,19). The *RAD51* gene codes for a protein of 400 amino acids, interacting directly with the *RAD52* gene product. Transcription of the *RAD51* gene is induced by DNA damaging agents like X-rays and MMS and is also regulated during the mitotic cell-cycle and meiosis. Structural similarity has been observed between the *RAD51* and *RAD57* proteins and with *DMC1*, a meiosis-specific protein in yeast (20,21). The *RAD51* protein also shares moderate homology with the ATP-binding domain of the bacterial strand exchange protein *RecA* (19). The *RAD51* protein is also similar to *RecA* in its DNA binding properties, suggesting structural and functional similarity between both proteins.

* To whom correspondence should be addressed at: Wassenaarseweg 72, 2333 AL Leiden, The Netherlands

We are interested in determining if genes belonging to the *RAD52* recombinational repair group are unique for *S.cerevisiae* or if this repair pathway is also conserved in evolution. As a starting we used the *RAD51* gene of *S.cerevisiae* as a heterologous probe on *S.pombe* DNA. Phylogenetic studies on the basis of 5S ribosomal RNA homologies indicate that *S.pombe* is evolutionary closer to man than to *S.cerevisiae* (22). Studies on DNA-repair genes in *S.pombe* can provide information on important domains in gene products, and on the evolutionary conservation of DNA-repair pathways (15,23).

In this article we report the isolation and molecular characterization of the *RAD51* homologue from *S.pombe*. The predicted protein shares extensive sequence similarity with the *S.cerevisiae RAD51* protein.

METHODS

Strains and growth conditions

The *S.pombe* diploid strain sp.101 (*leu1.32/leu1.32, ade6.704/ade6.704, ura4.D18/ura4.D18* (24)) was used for the gene deletion/disruption experiments. Growth conditions and media were previously described (25). The genomic *S.pombe* library and the plasmid *S.pombe* cDNA library used for the PCR reactions were kindly provided by M.H.M.Koken and P.Nurse, respectively.

General procedures

Chromosomal DNA was isolated from *S.pombe* cells essentially as described (26). Total RNA was purified according to Kaüfer *et al.* (27). Poly(A)$^+$ RNA was prepared by oligo(dT) selection using the PolyATtract method (Promega). Standard manipulations of nucleic acids were performed according to established procedures (28).

Heterologous hybridizations

PCR primers for the preparation of two flanking probes of the *S.cerevisiae RAD51* gene were designated as follows: The 5′-end probe was constructed with sense primer: 5′-GGAAACGGCAACGGTAGCAG-3′ (nucleotides 106 to 125) and anti-sense primer: 5′-GGAATTTGGCATGTCACGGC-3′ (nucleotides 614 to 595). The 3′-end probe was constructed with sense primer: 5′-TTGGATATTGGTGGCGGTGA-3′ (nucleotides 616 to 635) and anti-sense primer: 5′-GCGTG-GTGGAAGAATGTGCC-3′ (nucleotides 1069 to 1050). PCR was performed using 1 ng of plasmid YEp13-RAD51-23 as template (29). This plasmid contains the coding region of the *RAD51* gene, located between nucleotide 1 and 1200 (18). To avoid contamination with plasmid sequences, 1 μl from the amplification-mixture was used for a re-amplification. Finally, the PCR products were purified by agarose gel electrophoresis.

Heterologous hybridizations were performed in 5×SSPE, 5×Denhardt's, 0.5% SDS at 58°C. Filters were washed in 5×SSPE, 0.1% SDS (2×20 min), 2×SSPE, 0.1% SDS (2×20 min) at 58°C and finally rinsed in 2×SSPE at room temperature.

Sequence analysis

Genomic fragments were subcloned in pUC119 and 120 or in M13mp18 and 19 vectors (28,30). If necessary, unidirectional deletions were generated using the Erase-a-Base method (Promega). The nucleotide sequence was determined on both strands by the dideoxy chain termination method using T7 polymerase (31). In some cases sequencing reactions were carried out using the AutoRead sequencing kit (Pharmacia/LKB) and analyzed on an ALF automatic sequencer (Pharmacia/LKB).

Polymerase Chain Reaction

PCR was performed in 100 μl reactions containing 100 ng *S.pombe* genomic DNA or 10–50 ng DNA from a plasmid cDNA library, 50 pmol of both primers, 10 μl 10×AMP buffer (100 mM Tris.HCl (pH 8.3), 15 mM $MgCl_2$, 500 mM KCl, 0.1% gelatine), 10 μl 2.5 mM dNTPs and 2.0 units Taq Polymerase (Cetus). The PCR conditions were 30 cycles of 93°C (1 min), 50°C (1 min) and 72°C (3 min).

Gene disruption/replacement

The 0.4 Kb *EcoRI* fragment within the *rhp51*$^+$ coding region was deleted and replaced with a 1.7 Kb *EcoRI* fragment containing the *S.pombe ura4* gene (32), generating a 6.1 Kb *XhoI* fragment. The mutagenised *XhoI* fragment was isolated and used to transform a diploid *S.pombe* strain (sp.101). Transformation of yeast cells was performed according to the spheroplast method of Beach *et al.* (33). Diploid ura$^+$ transformants were selected and purified. Disruption of one of the two copies of the *rhp51*$^+$ gene was verified by blot-analysis. An h^{90}/h^+ derivative was then selected and used to generate ura$^+$ haploid spores.

Cell survival

Exponentially growing haploid cells ($O.D_{550}$= 0.5) were collected by centrifugation and resuspended in 0.1 vol. YES medium. Cell suspensions were irradiated with X-rays at a dose rate of 20 Gy/min (100 KV, 0.78 mm Al filter). Appropriate dilutions from wild-type and mutant cells were plated on YES agar and the survival was determined by counting the plates after 3 days of incubation at 29°C. Three plates were used for each dose. For UV survival experiments appropriate dilutions were plated on YES agar. The plates were immediately irradiated in a 'Stratalinker' (Stratagene) and colonies were counted after 3 days of incubation at 29°C.

Nucleotide accession number

The nucleotide sequence of the *rhp51*$^+$ gene is listed in the GenBank/EMBL under accession number: Z22691.

RESULTS

Isolation of the *Schizosaccharomyces pombe rhp51*$^+$ gene

To identify *RAD51* homologous sequences in *S.pombe* we applied the junction probe strategy described previously (10).

Southern blots containing *S.pombe* genomic DNA were probed with two flanking PCR fragments derived from the 5′-end and the 3′-end of the coding region of the *S.cerevisiae RAD51* gene. If an extended area of homology exists in *S.pombe*, there is a reasonable chance that both probes hybridize to the same restriction fragment: the junction fragment. Under low stringency conditions junction fragments were observed in *HindIII*, *SacI* and *XhoI* digests of *S.pombe* DNA (see Figure 1a, b). The sizes of the junction fragments are 5.4 Kb, 4 Kb and 4.8 Kb, respectively. These hybridization results strongly suggest the presence of a *RAD51* related gene in *S.pombe*.

To isolate the *S.pombe* homologue of *RAD51*, *rhp51*$^+$, a genomic lambda library (6) was screened with the 5′-and 3′-end probes. Four double positive clones were studied in more detail. Three of these clones contained the 4.8 Kb *XhoI* junction

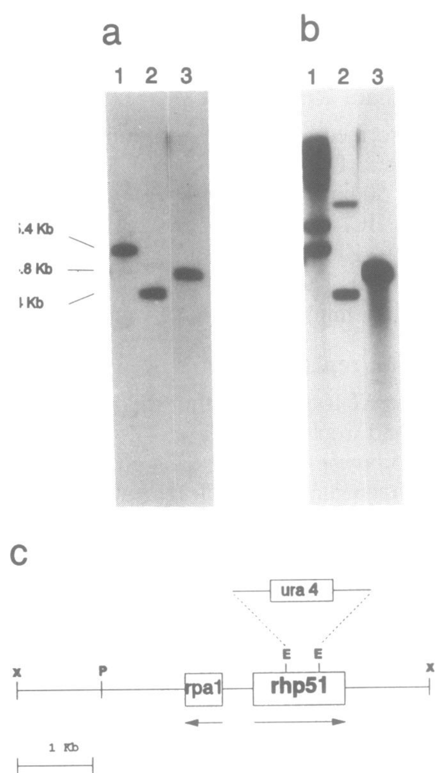

Figure 1. Southern blot hybridization of *S.pombe* genomic DNA with *RAD51* probes. **(a)** Hybridization with a 5'-end probe. **(b)** Hybridization of a duplicate filter with a 3'-end probe. Restriction enzymes used are: *HindIII* (lane 1); *SacI* (lane 2); *XhoI* (lane 3). **(c)** restriction enzyme map of the *rhp51*$^+$ region. The position of the ORFs of *rhp51*$^+$ and *rpaI* (ribosomal protein), is indicated as well as the direction of translation. The region replaced by the *ura4* fragment in the gene disruption/replacement experiment is also indicated. E=*EcoRI*; P=*PstI*; X=*XhoI*.

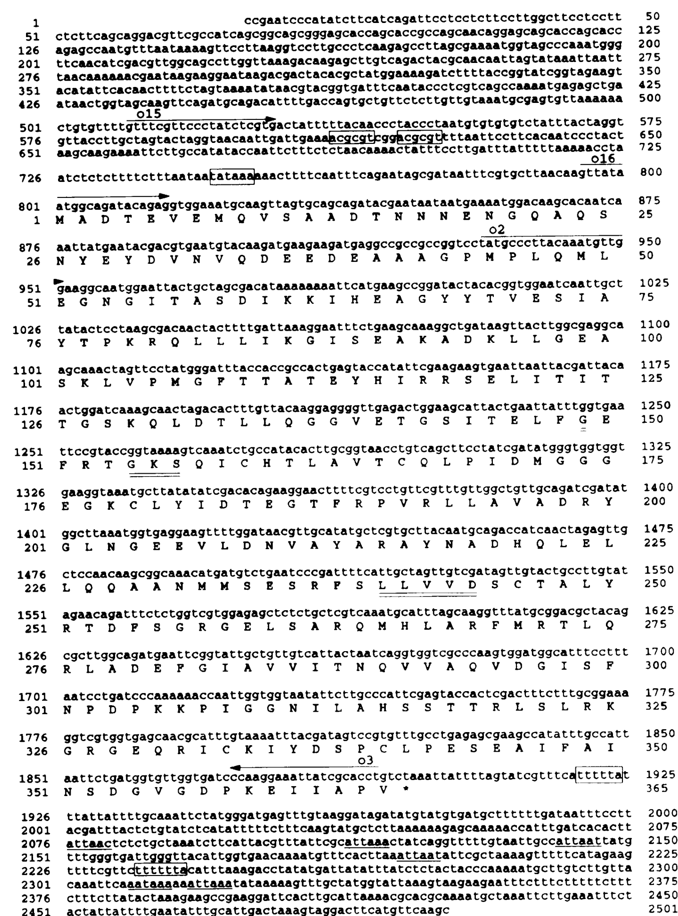

Figure 2. Nucleotide sequence of the *rhp51*$^+$ gene and the predicted amino acid sequence of the protein product. A-and B-type nucleotide binding sequences are double underlined. A putative TATA motif and *MluI* restriction sites (ACGCGT) are boxed. Putative termination signals for mRNA synthesis (TTTTTA) are boxed and polyadenylation sequences (A T/A TAA T/A/C) are underlined. The position of the oligonucleotides used for PCR are overlined with the 5'-3' direction given by internal arrows.

fragment. A preliminary restriction map of this fragment is shown in Figure 1c.

The chromosomal location of the *rhp51*$^+$ gene was determined by blot-analysis of *NotI* digested *S.pombe* genomic DNA. *NotI* digestion results in 17 fragments which can be seperated by pulse-field gel electrophoresis. The order of the fragments is known for each of the three chromosomes (34). Using as probe, the PCR fragment generated with primer o2 and o3 (see Figure 2), hybridization was observed with the 500 Kb *NotI* fragment H on chromosome I (results not shown). The chromosomal localization of the *rhp51*$^+$ gene was confirmed by probing filters containing DNAs from cosmid, phage P1 and YAC contigs (35).

Characterization of the rhp51 gene

Blot-analysis indicated that a small 0.4 Kb *EcoRI* fragment is located within the *rhp51*$^+$ gene. The nucleotide sequence of a region of 2.5 Kb encompassing this small *EcoRI* fragment was determined in both directions. The complete sequence of the *rhp51*$^+$ gene and flanking regions is shown in Figure 2. A long open reading frame (ORF) was observed between nucleotide positions 801 and 1895. Between nucleotide 484 and 573 another small ORF could be identified in the same orientation. The long ORF encodes a putative protein of 365 amino acids with a predicted molecular weight of 40 Kda. Comparison of this protein with the amino acid sequence of *RAD51* revealed 69% identical and 14% similar residues (see Figure 3). Most of the discrepancies were at the N-terminal end of the genes, as has also been found with other DNA-repair genes (8,36).

The absence of introns in the ORF between nucleotide 801 and 1895, was confirmed by amplification of *rhp51*$^+$ sequences in a cDNA library constructed in pREP1 (37). The position of the primers is given in Figure 2. PCR products of similar length were detected by blot-hybridization with both genomic DNA and cDNA using primer sets o2-o3 or o16-o3 (see Figure 4, lanes 1,2,3 and 7,8,9 resp.). No PCR product was obtained from cDNA using primer set o15-o3 (Figure 4, lanes 5 and 6) indicating that the small ORF between nucleotide 484 and 573 is not included in the *rhp51*$^+$ transcript.

To detect *rhp51*$^+$ specific transcripts, poly(A)$^+$ RNA was isolated from exponentially growing cells. Using a single-stranded probe, hybridization was detected with three distinct RNAs of 1.9 Kb, 1.6 Kb and 1.3 Kb (see Figure 5).

A computer search for the presence of known *S.pombe* genes within the 2.5 Kb region, using the BLAST program, revealed homology with the *S.pombe* gene coding for the acidic ribosomal protein *rpaI* (38). The start of translation of this gene is located

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rhp51    1 MADTEVEMQVSAADTNNN-------------ENGQAQSNYEYDVNVQDEE 37
           :     ..  :  : : :             . :  :   : . ...::
RAD51   22 MSTVPADLSQSVVDGNGNGSSEDIEATNGSGDGGGLQEQAEAQGEMEDEA 71

recA                                          1 MAIDENKQKALA 12
                                                 .:    .:  :
rhp51   38 -DEAAAGP-MPLQMLEGNGITASDIKKIHEAGYYTVESIAYTPKRQLLLI 85
            :::: :  .:.. :. ::::  :.::..: :  : : .:: :...:: :
RAD51   72 YDEAALGSFVPIEKLQVNGITMADVKKLRESGLHTAEAVAYAPRKDLLEI 121

recA    13 AALGQ--IEKQFGKGS-IMRLG-------EDRSMDVETISTGSLSLDIAL 52
            ....   .: .: .: .. .:         :  .. :: :::  ::  :
rhp51   86 KGISEAKADKLLGEASKLVPMGFTTATEYHIRRSELITITTGSKQLDTLL 135
           :::::::::::: :: .:::::: :: ..:.:::::: .::::: :::::
RAD51  122 KGISEAKADKLLNEAARLVPMGFVTAADFHMRRSELICLTTGSKNLDTLL 171

reca    53 GAGGLPMGRIVEIYGPESSGKTTL--TLQV-----IAAAQREGKTCAFID 95
             ::.  : : :..:    ::  .  :: :     :  .  ::: : .::
rhp51  136 -QGGVETGSITELFGEFRTGKSQICHTLAVTCQLPIDMGGGEGK-CLYID 183
             :::::::::::::::::::::.:::::::::.:.:.:::::: :::::
RAD51  172 -GGGVETGSITELFGEFRTGKSQLCHTLAVTCQIPLDIGGGEGK-CLYID 219

recA    96 AEHALDPI----YARKLGVD----IDNLLCSQPDTGEQALEI---CDALA 134
            :  . :.     :.  :..    .::. .  .  ... ::.      .
rhp51  184 TEGTFRPVRLLAVADRYGLNGEEVLDNVAYARAYNADHQLELLQQAANMM 233
           ::::::::::. .:.:.::. .. :.:::::::::::::: ::. :: ::
RAD51  220 TEGTFRPVRLVSIAQRFGLDPDDALNNVAYARAYNADHQLRLLDAAAQMM 269

recA   135 RSGAVDVIVVDSVAALTPKA-EIEGEIGDSHMGLAARMMSQAMRKLAGNL 183
             .   ..::::  ::        ::..  .: ::  :    . .::...
rhp51  234 SESRFSLLVVDSCTALYRTDFSGRGELSARQMHLARFM--RTLQRLADEF 281
           :::::::.::::  :::::::::::::::::::::.::  : ::::::.:
RAD51  270 SESRFSLIVVDSVMALYRTDFSGRGELSARQMHLAKFM--RALQRLADQF 317

recA   184 KQSNTLLIFINQIRMKIG-VMFGNPET--TTGGNALKFYASVRLDIR--- 227
                 ... ::.   ..   .  ::.     ::: :      :: .:
rhp51  282 ---GIAVVITNQVVAQVD-GISFNPDPKKPIGGNILAHSSTTRLSLRKGR 327
              :.:::.::::::::: :. :::::::::::::.::::::::...::.
RAD51  318 ---GVAVVVTNQVVAQVDGGMAFNPDPKKPIGGNIMAHSSTTRLGFKKGK 364

recA   228 ---RIGAVKEGENVVGSETRVKVVKNKIAAP 255
              ::  . ..  . .::    .  . .. :
rhp51  328 GEQRICKIYDSPCLPESEAIFAINSDGVGDPKE 360
           : ::.::. ::::::: : .:::  ::::::.:
RAD51  365 GCQRLCKVVDSPCLPEAECVFAIYEDGVGDPRE 397
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Figure 3. Comparison of the protein sequences of *S.pombe rhp51*$^+$, *S.cerevisiae RAD51* and *E.coli recA*. Protein sequences were aligned using the BESTFIT program. Identical amino acids between *recA*, *rhp51*$^+$ and *RAD51* are indicated by two lines (¦). Physicochemically similar residues are indicated by two dots (:). The ATP-binding sequences are underlined.

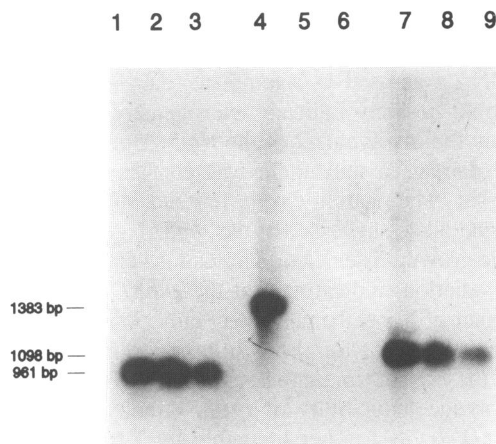

Figure 4. Southern blot analysis of *rhp51*$^+$ PCR products. Lane 1, 4 and 7: genomic *S.pombe* DNA. Lane 2,5 and 8: 10 ng *S.pombe* cDNA. Lane 3,6 and 9: 50 ng *S.pombe* cDNA. Lane 1,2 and 3: primer set o2-o3. Lane 4,5 and 6: primer set o15-o3. Lane 7,8 and 9: primer set o16-o3. For primer positions see Figure 2. The genomic fragment located between primer o2 and o3 was used as a probe.

at position 455 and the gene is transcribed in the opposite direction in comparison with *rhp51*$^+$. Several other repair genes in *S.pombe* are also closely linked to genes coding for ribosomal proteins (24,39).

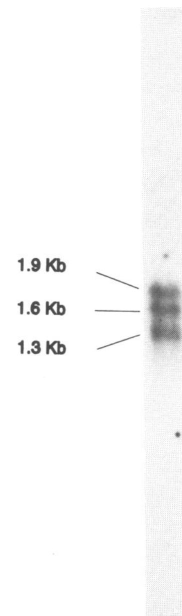

Figure 5. Northern analysis of *rhp51*$^+$ transcripts. 5 μg poly(A)$^+$ RNA was fractionated on a 1% agarose gel containing formaldehyde, blotted and hybridized with an anti-sense probe derived from the 0.4 Kb *EcoRI* fragment.

Gene disruption/replacement

To determine whether the *rhp51*$^+$ gene is involved in the repair of X-ray damage, a null allele was constructed by homologous recombination. A plasmid was made in which the small *EcoRI* fragment between nucleotide 1248 and 1638 was replaced by a 1.7 Kb fragment containing the *ura4* gene from *S.pombe* (see Figure 1c). The constructed 6.1 Kb linear *XhoI* fragment was used to transform a diploid *S.pombe* strain to uracil prototrophy. An h^{90}/h^+ derivative was used to generate haploid spores. After plating, 50% of the colonies were ura$^+$ and 50% ura$^-$. All ura$^+$ colonies were radiation sensitive. Blot-analysis showed that the selected ura$^+$ haploid contains the disrupted *rhp51*$^+$ allele (results not shown).

The slow growth of the haploid mutant indicates that the mutation effects the growth rate but not the viability of the mutant. Survival experiments show that the *rhp51*$^+$ null mutant is extremely sensitive to ionizing-radiation (see Figure 6A). The radiation dose giving 50% survival (LD_{50}) for the *rhp51*$^+$ mutant is 14 Gy, compared with 260 Gy for wild-type cells. The UV sensitivity of the *rhp51*$^+$ null mutant is illustrated in Figure 6B. As is evident from this figure, the *rhp51*$^+$ mutant is also sensitive to UV light.

DISCUSSION

Here we present the isolation of the *rhp51*$^+$ gene from the fission yeast *Schizosaccharomyces pombe*, which is involved in the repair of DNA-damage induced by ionizing-radiation. Screening of a genomic *S.pombe* library with 5′-end and 3′-end probes derived from the *S.cerevisiae RAD51* gene, resulted in the isolation of several double-positive phage clones. Using this so-called junction-probe strategy, the isolation of sequences with only marginal homology is avoided (10).

Sequence analysis revealed an ORF between nucleotide 801 and 1895 encoding a protein of 365 amino acid residues. The

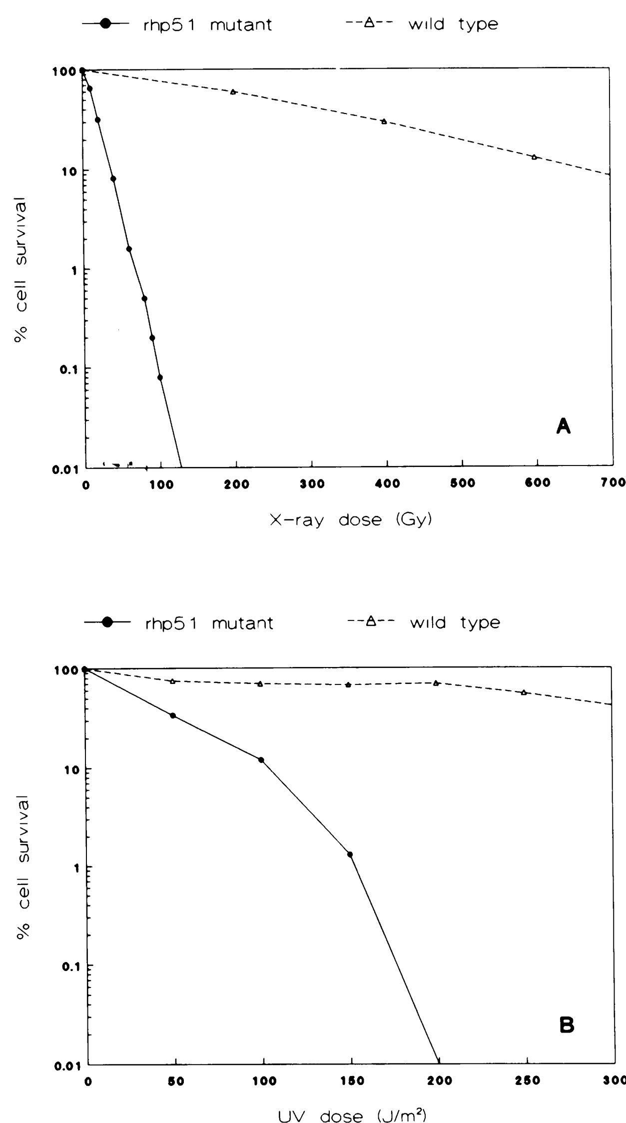

Figure 6. Survival following irradiation with X-rays (**A**) and UV light (**B**) of cells from a haploid *rhp51*$^+$ null mutant and from a haploid wild-type *S.pombe* strain.

sequence context of the start codon CAAGTTATAATGG has only one mismatch with the eukaryotic consensus sequence for start codons (a G at positions −9, −6 and +4 and a purine at −3) (40). The region between nucleotide 801 and 1895 is not spliced since PCR analysis of *rhp51*$^+$ sequences in genomic DNA and cDNA resulted in products of similar length (Figure 4, lanes 7,8,9).

Analysis of the nucleotide sequence upstream of the coding region of the *rhp51*$^+$ gene revealed a TATA motif at position 746 and two cleavage sites for the restriction enzyme *MluI* at position 612 and 621. In *S.cerevisiae* these so called 'MluI cell-cycle boxes' or MCB's are involved in both the periodic expression of genes at the G1/S border of the cell-cycle and the coordinate regulation of DNA synthesis genes (31). In most genes these elements are located between position −90 and −250 with respect to the ATG start codon. In the promotor region of the cell-cycle regulated *S.cerevisiae RAD51* gene, there are also two *MluI* sites. The presence of *MluI* sites in the *rhp51*$^+$ upstream region may therefore suggest a cell-cycle regulated expression of this gene. The '*MluI* activating system' is conserved in *S.pombe*, but it is far more limited in extent. *Cdc22*, which is expressed at the G1/S boundary, is the only gene known so far to contain functional MCB-boxes (41).

The deduced amino acid sequence of the *rhp51*$^+$ gene strongly resembles the *RAD51* sequence. The overall homology of the two proteins is 69% identical and 14% similar amino acid residues. Except for the N-terminal region, the alignment of the sequences is almost continuous throughout both proteins (see Figure 3). The homology is most extensive in the middle part and the carboxyl-terminal region: 78% identity between aa 85 and aa 365 of *rhp51*$^+$. The A-type ATP-binding sequence, GXXXXGKS, at position 149 of *rhp51*$^+$ is identical with the sequence found in *RAD51*. The B-type ATP-binding sequence, LLVVD, has only one mismatch with the consensus observed in *RAD51*: isoleucine at position 277 in *RAD51* is replaced by leucine, at position 241 of *rhp51*$^+$. The N-terminal region of *rhp51*$^+$ between amino acid 1 and 85 is only 40% identical with *RAD51*. The *rhp51*$^+$ protein is also 22 amino acids shorter at the N-terminus in comparison with the *RAD51* gene product.

The core region of the *RAD51* protein between amino acid residues 110 and 400 and the bacterial *RecA* protein extending from amino acid 1 to 255, display 29% identity and an additional 24% similarity. Within this region of the *RecA* protein the same percentage of homology was found with the *rhp51*$^+$ gene product: 29% identical amino acids and an additional 24% similar amino acids between amino acid 73 and 360 of *rhp51*$^+$.

Northern-blot analysis showed the presence of three *rhp51*$^+$ specific transcripts of 1.9 Kb, 1.6 Kb and 1.3 Kb. These three transcripts are presumably the results of different transcription initiation and/or termination sites. In the 3′-untranslated region two putative transcription termination signals were identified at position 1919 and 2235, as well as six putative polyadenylation signals at position 2076, 2113, 2141, 2195, 2309 and 2317. Amplification reactions using sense primers located in the region upstream of the *rhp51*$^+$ ORF did not result in specific products when cDNA was used as a template (data not shown). These results could possibly indicate heterogeneity at the 3′-end.

To study the involvement of the *rhp51*$^+$ protein in the repair of X-ray damage a null allele was constructed by disruption mutagenesis. The viability of a haploid strain carry ing the mutagenized allele suggests that the *rhp51*$^+$ gene is not essential for mitotic growth. The deletion mutant is very sensitive towards ionizing-radiation, indicating that the *rhp51*$^+$ gene is required for the repair of X-ray damage (see Figure 6A). The dose giving 50% survival (LD_{50}) for the wild-type strain is 260 Gy. The LD_{50} for the *rhp51*$^+$ mutant is only 14 Gy. Preliminary results on the damage inducibility of *rhp51*$^+$ indicate that the gene, unlike *RAD51* (18), is not inducible by X-rays. UV survival experiments show that the *rhp51*$^+$ mutant strain is also very sensitive to UV light (see Figure 6B). These results suggest that, in contrast to *S.cerevisiae*, part of the UV-induced damage in *S.pombe* is repaired by recombinational repair pathways. However, the slow growth of the *rhp51*$^+$ deletion mutant may indicate that these cells are sick, and therefore the killing observed with UV light may not all reflect the repair deficiency in these cells.

The structural and functional similarity of the *rhp51*$^+$ and *RAD51* genes suggest that certain components of the repair of DSB's have been conserved among yeast strains. The recent identification of *RAD51/rhp51*$^+$ homologues in man and chicken

indicates that the DSB repair pathway is also conserved in higher eukaryotes (42,43).

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